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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**



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| Application of | : | Kohn, *et al.* |
| Serial No. | : | 08/225,478 |
| Filed: | : | April 8, 1994 |
| For | : | Gene Therapy by Administration of Genetically Engineered CD34+ Cells Obtained From Cord Blood |
| Group | : | 1804 |
| Examiner | : | Milne |

Assistant Commissioner of Patents
Washington, D.C. 20231

Cancelled 11/26/97 (D)

**DECLARATION UNDER 37 CFR 1.132**

I, Donald B. Kohn, hereby declare as follows:

1. That I am a co-inventor of the claimed subject matter of the above-identified application;

2. That I am a co-author of Kohn, *et al.*, Nature Medicine, Vol. 1, No. 10, pgs. 1017-1023 (October 1995), and attached hereto as Exhibit 1;

3. That Exhibit 1 describes work done by me, my co-inventors, or others acting on our behalf;

4. That such work includes that described in Paragraphs 5 through 13 hereinbelow;

5. That CD34+ cells were isolated from the cord blood of a newborn infant patient, hereinafter referred to as "the patient," diagnosed with adenosine deaminase (ADA) deficiency;

6. That the isolated CD34+ cells were transduced with the retroviral vector LASN, which includes cDNA encoding normal human adenosine deaminase under the control of a Moloney Murine Leukemia Virus LTR and a neomycin resistance gene under the control of an SV40 promoter;

7. That the transduced CD34+ cells then were returned to the patient by intravenous infusion;

8. That, at age 18 months, CD34+ bone marrow cells were isolated from the patient and selected for G418 resistance;

9. That cells found to be G418 resistant were grown in culture for two weeks;

10. That CD34+ cells from the bone marrow of a normal donor were transduced with the retroviral vector LN, which includes a neomycin resistance gene under the control of the Moloney Murine Leukemia Virus LTR, and the cells then were cultured in the presence of G418 to select for G418 resistance;

11. That the cells described in Paragraph 9, obtained by culturing selected G418 resistant CD34+ cells obtained from the patient, and the G418 resistant cells described in Paragraph 10, obtained from a normal donor, were evaluated for expression of adenosine deaminase;

12. That 24,051.0 nmol of ADA per hour per mg of protein was expressed by the cells in the culture obtained by culturing G418 resistant CD34+ cells obtained from the patient;

13. That 4,694.0 nmol of ADA per hour per mg of protein was expressed by the LN vector transduced cells obtained from the bone marrow of the normal donor;

14. That, in my opinion, the results shown in Paragraphs 12 and 13, which also are shown in Table 3 of Exhibit 1, show that the level of ADA produced by the LASN vector transduced CD34+ cells from the patient is similar to that of a normal person, and therefore, such results provide evidence, although indirect, that the patient's cells which include the LASN vector are expressing ADA at levels sufficient to provide a therapeutic effect;

15. That I also am a co-author of an abstract by Kohn, et al., entitled "Selective Accumulation of ADA Gene-Transduced T-Lymphocytes Upon PEG-ADA Dosage Reduction After Gene Therapy with Transduced CD34+ Umbilical Cord Blood Cells," said article having been published in Blood, Vol. 86, No. 10, Supp. 1, Abstract 1168 (November 15, 1995), and attached hereto as Exhibit 2;

16. That Exhibit 2 describes work done by me, my co-inventors, or others acting on our behalf;

17. That such work includes that described in Paragraphs 18 through 20 hereinbelow;

18. That three newborn infant patients diagnosed with ADA deficiency each were given infusions of autologous umbilical cord blood CD34+ cells that had been transduced with the retroviral vector LASN;

19. That each of the three patients also received PEG-ADA in an amount of 60 units per kilogram per week;

20. That, at 18 months of age, the dosage of PEG-ADA for each patient was reduced to 20 to 30 units per kilogram per week;

21. That those skilled in the art, when treating ADA deficiency, administer PEG-ADA to a patient in an amount of at least 30 units per kilogram per week to about 60 units per kilogram per week in order to achieve a therapeutic effect in the patient;

22. That after lowering the dosage of PEG-ADA to 20 to 30 units per kilogram per week in each patient, each patient remained in good health and no changes in the clinical conditions of the patients were observed;

23. That subsequent to the publication of Exhibit 2, and at 30 months of age, the dosage of PEG-ADA administered to one of the patients was lowered to 15 units per kilogram per week;

24. That after lowering the dosage of PEG-ADA to 15 units per kilogram per week in one of the patients, such patient remained in good health and no changes in the clinical condition of the patient were observed;

25. That I hereby declare that all statements made herein are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: March 3 1997

Donald B. Kohn